Published in "Journal of Ecology doi: 10.1111/1365-2745.12253, 2014"
which should be cited to refer to this work.

Differential allocation and deployment of direct and indirect defences by *Vicia sepium* along elevation gradients

Sergio Rasmann[1,2], Aline Buri[1], Marie Gallot-Lavallée[1], Jessica Joaquim[1], Jessica Purcell[1] and Loïc Pellissier[3]*

[1]*Department of Ecology and Evolution University of Lausanne, Biophore Building, 1015 Lausanne, Switzerland;* [2]*Department of Ecology and Evolutionary Biology University of California, 321 Steinhaus Hall, Irvine, CA 92697, USA; and* [3]*Department of Biology Unit of Ecology & Evolution University of Fribourg, Ch. du Musée 10, CH-1700 Fribourg, Switzerland*

Summary

1. Dissecting drivers of plant defence investment remains central for understanding the assemblage of communities across different habitats. There is increasing evidence that direct defence strategies against herbivores, including secondary metabolites production, differ along ecological gradients in response to variation in biotic and abiotic conditions. In contrast, intraspecific variation in indirect defences remains unexplored.

2. Here, we investigated variation in herbivory rate, resistance to herbivores and indirect defences in ant-attracting *Vicia* species along the elevation gradient of the Alps. Specifically, we compared volatile organic compounds (VOCs) and ant attraction in high- and low-elevation ecotypes.

3. Consistent with adaptation to the lower herbivory conditions that we detected at higher elevations in the field, high-elevation plants were visited by fewer ants and were more susceptible to herbivore attack. In parallel, constitutive volatile organic compound production and subsequent ant attraction were lower in the high-elevation ecotypes.

4. We observed an elevation-driven trade-off between constitutive and inducible production of VOCs and ant attraction along the environmental cline. At higher elevations, inducible defences increased, while constitutive defence decreased, suggesting that the high-elevation ecotypes compensate for lower indirect constitutive defences only after herbivore attack.

5. *Synthesis.* Overall, direct and indirect defences of plants vary along elevation gradients. Our findings show that plant allocation to defences are subject to trade-offs depending on local conditions, and point to a feedback mechanism linking local herbivore pressure, predator abundance and the defence investment of plants.

Key-words: alpine, ant, extrafloral nectar, herbivory, mutualism, myrmecophily, natural enemy, plant–herbivore interactions

Introduction

Insect herbivores and plants together comprise more than half of the terrestrial macrobiodiversity described today (Strong, Lawton & Southwood 1984), and nearly half of existing insects species are phytophagous, consuming vegetable material including leaves, stems, roots, flowers, nectars and pollen (Schoonhoven, van Loon & Dicke 2005). To counter insect aggression, plants have evolved defence systems, classically grouped into two categories. The first type, called 'direct defences', includes chemical or mechanical defences, and physiological adaptations that limit plant palatability to insect herbivores (Schoonhoven, van Loon & Dicke 2005). The second type, called 'indirect defences', includes the plant's active attraction of a partner at a higher trophic level, such as the predators or parasitoids that consume or deter the herbivores (Turlings & Wäckers 2002; Dicke & Baldwin 2010). Attraction of carnivorous insects is enhanced by the plant production of alimentary rewards like food bodies or extrafloral nectaries (EFNs) (Heil 2008; Kessler & Heil 2011), or information-rich cues such as the production of volatile organic compounds (VOCs) (Turlings, Tumlinson & Lewis 1990;

*Correspondence author. E-mail: loic.pellissier@unifr.ch

Dicke & Baldwin 2010), both of which have been shown to limit herbivory (Heil *et al.* 2001; Kessler & Baldwin 2001) and increases plant fitness (Stephenson 1982; de la Fuente & Marquis 1999; Schuman, Barthel & Baldwin 2012).

Plants may employ their defence systems either constitutively or only in response to leaf damage by herbivores (= induced defences) (Karban & Baldwin 1997). Particularly, plant induction of defences by chewing herbivores is orchestrated by the synthesis of jasmonic acid, which is produced from linolenic acid through the octadecanoid-signalling pathway and generally, results in increased production of direct and indirect defences (Howe & Jander 2008). It has been postulated that inducible defences originated as a cost-saving strategy (Karban & Baldwin 1997; Agrawal & Karban 1999). As induced and constitutive defences utilize the same pool of resources, they should negatively correlate with each other (Thaler & Karban 1997; Heil *et al.* 2004; Rasmann *et al.* 2011). Following the same reasoning, direct and indirect defences also are expected to be negatively correlated and may be differentially selected under contrasting environmental conditions (Ballhorn *et al.* 2008). To date, relatively few studies have investigated intraspecific variation in indirect defence in natural populations of plants (Wason & Hunter 2014) and to our knowledge virtually, none have compared direct and indirect defences along environmental gradients.

The type of defence and the amount of energy a plant allocates should represent an optimal strategy given the local abiotic conditions (soil resources, climate, photosynthesis activity, etc.) and the abundance and identity of attackers (Coley, Bryant & Chapin 1985; Herms & Mattson 1992; Fine, Mesones & Coley 2004). Therefore, contrasted environmental conditions along gradients should influence plant/herbivore interactions. Abundance and composition of insect herbivore communities shift among habitats because ectotherms are affected by abiotic conditions (Novotny *et al.* 2005; Singer & Stireman 2005; Rodríguez-Castañeda *et al.* 2010; Pellissier *et al.* 2012a). Similarly, the abundances of natural enemies of herbivores should vary along environmental gradient potentially limiting the benefit of indirect defences (Machac *et al.* 2011; Rodriguez-Castaneda *et al.* 2011). For instance, ants are frequently involved in plant indirect defences (Beattie 1985), as they are the dominant insect predators in many different ecosystems (Holldobler & Wilson 1990). However, this defence system is dependent on ants being available and therefore may be counter-selected in environments with low ant frequencies (Rodriguez-Castaneda *et al.* 2011; Reymond *et al.* 2013). In addition, variation in environmental conditions may influence plant metabolic activity and the extent to which defences can be synthesized. For instance, due to the differential costs of tissue replacement in low- and high-resource environments, the optimal defence allocation would be higher in low-resource habitats and lower in high-resource habitats (Coley, Bryant & Chapin 1985; Fine, Mesones & Coley 2004). Therefore, along environmental gradients, the distribution and abundance of herbivorous insects and thus the probability of attack is assumed to be non-uniform (Hodkinson 2005; Pellissier *et al.* 2012a) and the plant investment in direct and indirect defences is expected to form clines.

Elevation gradients are optimal systems for inferring shifts in species interactions in varying environmental conditions (Pickett 1989; Körner 2007; Beier *et al.* 2012; Rasmann, Alvarez & Pellissier 2014). Indeed, elevation gradients act as 'natural experiments' by providing variation in abiotic factors under which biotic interactions can be evaluated (Preszler & Boecklen 1996; Darrow & Bowers 1997; Salmore & Hunter 2001; Zehnder *et al.* 2009). Compared with much large-scale latitudinal gradients, elevation gradients minimize the confounding effects of historical and biogeographical differences in, for instance, plant and herbivore species pools (Hodkinson 2005). The shifts in abiotic and biotic conditions along elevation gradients may promote turnover in plant and insect strategies in communities. Plant defence strategies are, therefore, expected to differ along these gradients.

Specifically, it has been postulated that at high elevation, because of lower herbivory pressure linked to a decrease in herbivore abundance, plants would decrease their investment in primary defences (Pellissier *et al.* 2012a). In parallel, the need to attract a predator partner should decrease with increasing elevation (Rodriguez-Castaneda *et al.* 2011). On the other hand, as high-elevation plants might suffer from a reduction in metabolic activity and growth due to poorer soil quality and colder temperatures, we might predict an increase in low-cost inducible defences at high elevation. Using a combination of field observation, field experiments and common garden experiments, we here investigated potential variation in herbivory and direct resistance as well as bodyguard recruitment in *Vicia sp*. along elevation gradients. We measured ant attendance, and herbivory in the field, and compared VOCs production and ant attraction between high- and low-elevation ecotypes. We asked the following questions: (i) Is there variation in abundance and diversity of ant species visiting *V. sepium* across the plant elevation range? (ii) Is herbivory pressure decreasing with elevation, and in turn do we observe a lower resistance in the high-elevation ecotype? (iii) Does the amount of constitutive and induced VOCs production differ along elevation gradients and does this affect attraction of ants?

Materials and methods

FIELD OBSERVATION OF ANT VISITATION

Vicia sepium L. (Fabaceae) or bush vetch is a nitrogen-fixing, perennial, leguminous climbing plant that grows in hedgerows, grasslands, the edges of woodland, roadsides and rough ground across western Europe, Russia, the Caucasus, and where habitat is suitable, in Greenland. Its widespread continental distribution is also reflected in its wide elevation range in the Swiss Alps, in which it has been reported to grow from the lowlands up to the sub alpine zone. *Vicia sepium* is similar in appearance to the common vetch (*V. sativa* L.), but the common vetch is annual and only occurs in the lowlands.

To assess variation in ant attendance of the plant along elevation gradients, during the month of July 2012, we visited 26 populations of *V. sepium* situated between 490 m and 2200 m above sea level in the Swiss Alps. For each population, during its flowering stage, we recorded over an investigated surface of 50 by 50 m the number of

plant individuals with and without ants during a 30-min observation period. Observations were only conducted during warm and sunny conditions. Ants were collected and identified in the laboratory using morphological keys coupled with genetic barcoding. We computed the proportion of plants interacting with ants per population and related this to elevation with a General Linear Model (GLM) using a binomial distribution. The significance of the relationship was assessed using a Wald's *Z*-test. We also measured extrafloral nectar sugar concentration, however, because of strong variability in nectar droplets that were visible on the extrafloral nectaries, we could only measure nectar production from two populations along the same transect. Using a temperature-compensated refractometer (ATAGO-53, ATAGO USA, Inc., Bellevue, WA, USA), we measured 12 plants from one high population site (1980 m above sea level) and 12 plants from a low population site (850 m above sea level). Plants were separated from each other by at least 10 m.

HERBIVORY RATES IN COMMON GARDENS ALONG ELEVATION GRADIENTS

To study herbivore pressure on *Vicia sp.* along elevation gradients, we transplanted individuals of *V. sativa* to different elevations and exposed them to natural herbivory. As *V. sativa* occurs in lowland habitats, there is no pre-adaptation to variation of herbivores at different elevations, and thus, this species constitutes a good bioassay to measure herbivore attacks along the elevation gradient. *V. sativa* seeds (B & T World Seeds, Aigues-Vives, France) were germinated in regular potting soil (Orbo-2, Schweizer AG, Lausanne; Switzerland) with perlite (3:1). After 7 days, same-size plantlets were transplanted into 15-cm diameter plastic pots containing the same soil as for germination and placed in a greenhouse at 25/18 °C, 60% relative humidity, and a photoperiod consisting of 14 h of daylight. After 2 weeks of growth, 10 plants were placed in six different grasslands along an elevation gradient in the western Swiss Alps. Locations of the grasslands are the following: Lavey (440 m), Gryon (1090 m), Solalex (1470 m), Martinaux (1520 m), Anzeindaz (1870 m), Riondaz (2150 m). After four weeks of exposure to natural herbivory, damage was measured using the following ranking: (0) no damage, (1) small marks on the leaves, (2) leaflet partially eaten, (3) entire leaflets eaten, (4) entire leaves eaten, and (5) dead plant. We tested the relationship between elevation and damage using a linear mixed model accounting for the grouped effect of individuals within each site using the lme4 package and tested the significance with an *F*-test using the Kenward–Roger approximation.

ELEVATION EFFECTS ON DIRECT AND INDIRECT DEFENCES IN *V. SEPIUM*

To measure elevation effects on chemical defence production, resistance and ant attraction, we collected seeds from eight populations of *V. sepium* from low elevation (<800 m) and eight from high elevation (>1500 m) in the Swiss Alps. Seeds were scarified using sandpaper to stimulate germination, and all plants were grown in the greenhouse under the same soil, light, temperature and humidity conditions as above.

Plant direct resistance: We assessed the integrative outcome of *V. sepium* direct defences using a measure of resistance against a generalist chewing herbivore. We placed 10 first instar larvae of *Spodoptera littoralis* (obtained from Syngenta, Stein, Switzerland) on plants of *V. sepium* from high and low elevation wrapped with fine-meshed nylon netting to prevent larvae from leaving the plants. *S. littoralis* is a generalist caterpillar known to feed on plants of at least 40 different families (Brown & Dewhurst 1975) and widely used to perform plant resistance bioassays. As *S. littoralis* is not present in Switzerland, it has the advantage of removing potential confounding effects of pre-adaptation to plant species and ecotypes. After 10 days of feeding, we collected the caterpillars, froze them immediately in liquid nitrogen and weighed them. We compared the weight of herbivores grown on high- and low-elevation plants using an analysis of variance (ANOVA). We used a balanced random sample of seeds from each of the eight populations but did not track population affiliation in the experiment. As a consequence, while we detect differences between low- and high-elevation ecotypes irrespective of populations, we cannot evaluate the variation among populations from high- or low-elevation ecotypes. Cyanide-based secondary metabolites have classically been considered as chemical defences against herbivores in Fabaceae and have been measured in several *Vicia* species (Wink 2003), but to our knowledge, they have never been reported in *V. sepium.* To confirm this, we additionally quantified CN levels in *V. sepium* leaves of five control and five induced plants of high and low elevation using the Brinker and Seigler (1992) protocol.

Indirect defence: To characterize the VOCs emissions of *V. sepium*, we used a modified dynamic headspace collection method (Kessler & Baldwin 2001). Leaf volatiles were collected by placing an oven bag (1 L volume) around the plants, and by pulling air through the chamber into a charcoal filter trap (Orbo-32; Supelco, Bellefonte, PA, USA) with a flow rate of 250 mL min^{-1} for 4 h. Traps were eluted with 250 µL of dichloromethane after the addition of 250 ng of tetraline as an internal standard, and 2 µL aliquots were analysed using gas chromatography–mass spectrometry (GC/MS) with a Thermo Trace GC Ultra Lan GC system coupled with a quadrupole-typemass-selective detector (DSQ II from Thermo (Thermo Fisher Scientific Inc., Waltham, MA, USA; transfer line 220 °C, source 240 °C, ionization potential 70 eV). The sample was injected on an apolar column (Zebron ZB-5MS, 30 m, 0.25 mm internal diameter, 0.25 µm film thickness, Phenomenex, Torrance, CA USA). Helium at a constant flow (1.2 mL min^{-1}) was used for carrier gas flow. After splitless injection (250 °C), the column temperature was raised from 40 to 150 °C at 6 °C min^{-1}, then to 220 °C at 6 °C min^{-1} followed by a final stage of 5 min at 220 °C. Peak areas were integrated from selected ion chromatograms specific to the individual compounds and normalized by the peak area of the internal standard. Peaks were identified when possible by the comparison of retention times (RT) and mass spectra with standards. Volatile emissions are reported as nanograms tetraline equivalents per hour. We analysed constitutive volatile production of 10 uninduced (undamaged) plants and of 10 plants that were induced with jasmonic acid. Both the non-induced and induced treatments included five plants from high and five plants from low elevation that originated from different populations. Induction was carried out by placing cotton tips spiked with 5 µL methyl-jasmonate (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland, CAS Number 39924-52-2) near each plant, which was covered with 1L oven bag for 12 h. We induced plants with methyl-jasmonate, as it is known to activate plant defences against attacks by herbivores (Farmer & Ryan 1992). This approach provides a more standardized induction than using real herbivores, due to their intrinsic variation in herbivory levels between high- and low-elevation populations. Uninduced plants were covered with the oven bag, but the cotton tip was left without methyl-jasmonate. We compared the production (ng h^{-1}) of VOCs from the different RT between constitutive and induced plants from high and low elevation using a three-way ANOVA including as factors RT, jasmonic acid treatment and elevation of origin. To reach normality in the model residuals, we used a

square root transformation of the response variable. We also compared the total amount of VOCs by summing compounds from all RT between elevation and treatments using an ANOVA.

ANT ATTRACTION EXPERIMENT

We next assessed the difference in ant attraction of high- and low-elevation plants using custom-made glass olfactometers modified after Rasmann *et al.* (2005). The olfactometer is a two-sided system of glass tubes consisting of two vessels attached, on the opposite directions, to a central chamber by a 10-cm-long glass tube each. *Vicia sepium* plants were grown in common garden as described previously, and after three weeks of growth, the shoots of non-flowering *V. sepium* plants were introduced in the vessels on both sides of the olfactometer (Fig. 1). After placing five ants in the central chamber, they were allowed to freely walk through the tubes in the direction they choose for 20 min. The number of ants found in each side of the olfactometer was counted after this period. Olfactometers were washed with ethanol between uses to suppress potential ant trails in the tubes (Holldobler & Wilson 1990). We used several ant species (*Formica fusca, Formica selysi, Myrmica.* spp.) from colonies collected in the field to conduct this experiment as *V. sepium* is generalist in term of species attraction (see Results section). We performed a first experiment comparing constitutive attraction of ants between high- and low-elevation ecotypes of *V. sepium.* The second experiment was carried out by inducing the plants from both elevations with methyl-jasmonate as described earlier. Each treatment was replicated 20 times using the same selection of ant species. We compared ant attraction between high and low elevation with and without treatment with jasmonate using an ANOVA.

Results

ANT ATTENDANCE, NECTAR PRODUCTION AND HERBIVORY ALONG ELEVATION

We investigated 26 populations of *V. sepium* in the Swiss Alps ranging from 490 to 2200 m. We found a decrease of ant attendance with elevation (Fig. 2a, $Z = -2.387$, $P = 0.017$, Fig. 1). In lowland populations, almost all plant species were visited by ants. This ratio decreased with elevation so that a large proportion of individuals were not attended at high elevation. *V. sepium* individuals were attended by very different species of ants. Across the 26 population visited, we observed 13 species of ants (*Camponotus herculeanus*, *Formica cunicularia*, *Formica fusca*, *Formica paralugubris*, *Formica pratensis*, *Formica sanguinea*,

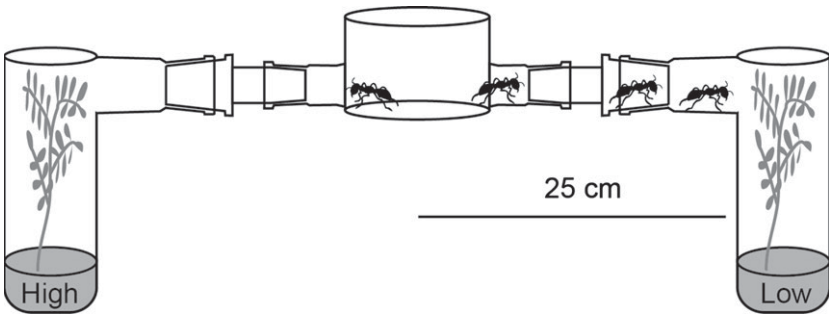

Fig. 1. Ant olfactometer. Shown is a modified six-arm olfactometer after Rasmann *et al.* (2005) that was used to measure ant attraction toward healthy or jasmonic acid–induced *Vicia sepium* plants, coming from high or low elevation in the Swiss Alps.

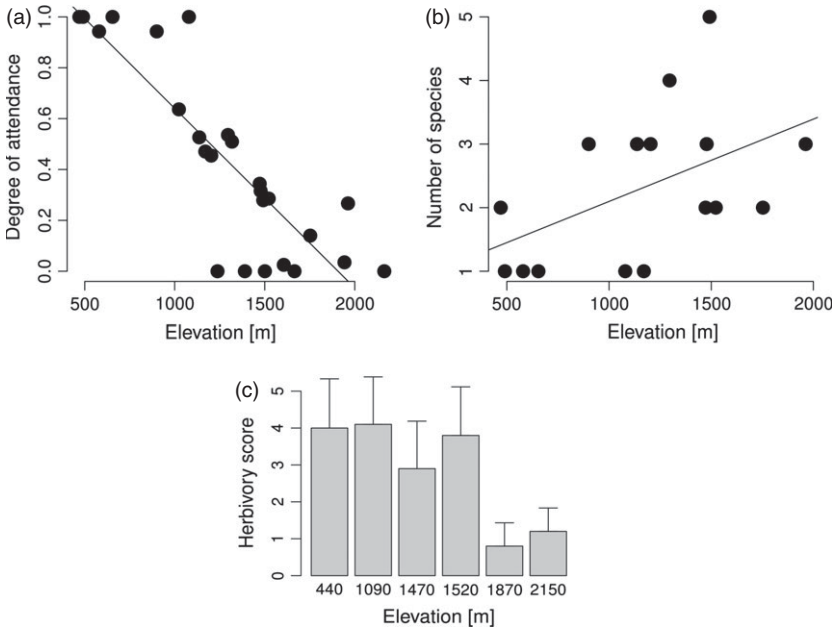

Fig. 2. Ant visitation and herbivory rates along elevation. Shown is the proportion of *Vicia sepium* plants attended by ants in natural populations spanning a large elevation gradient (a), the average number of ant species found attending plants in those populations (b) and the average (± SD) amount of herbivory in *Vicia sativa* placed at different elevations (c).

Myrmica sulcinodis, *Myrmica specioides*, *Lasius flavus*, *Lasius niger*, *Lasius umbratus*, *Plagiolepis* spp., *Solenopsis* spp.) interacting with *V. sepium,* demonstrating the generalist behaviour of the plant. Overall, there was a marginal tendency of populations at higher elevation to be visited by a larger number of species (Fig. 2b, $F_{1,14} = 4.4$, $P = 0.055$). Finally, when individuals of *V. sativa* were placed in grasslands along the elevation gradient, we observed a strong decrease in herbivory especially above an elevation of 1800 meters (Fig. 2c, $F_{1,107} = 7.58$, $P = 0.001$).

ELEVATION EFFECTS ON DIRECT AND INDIRECT DEFENCES IN *V. SEPIUM*

We found that the total weight of larvae growing on plants from high-elevation ecotypes was significantly higher than the weight of those growing on the low-elevation ecotypes (Fig. 3, $F_{1,22} = 5.4$, $P = 0.034$). This is partially due to the greater survival of larvae on the high-elevation ecotype. We expected differences in larval weight based on previous experiments (Pellissier *et al.* 2012a,b), but not a strong difference in survival. Thus, we conducted a first trial with 10 replicates and confirmed the results by performing a second trial with 20 replicates. We present the full results including the total 30 replicates (Fig. 3) and accounted for the experimental block effects in the model. We could not detect cyanogenic glycoside production in any of the *V. sepium* samples screened, either in control plants or in induced plants.

The VOCs collection analyses indicated that similar compounds were emitted by *V. sepium* plants from low and high elevation. The two ecotypes differed, however, in the amount of VOCs emitted (Fig. 4). In particular, the amount of VOCs differed among compounds from different RT ($F_{1,405} = 13.7$, $P < 0.0001$), but also between treatment with jasmonate ($F_{1,405} = 108.7$, $P < 0.0001$) and elevation ecotype ($F_{1,405} = 4.11$, $P = 0.04$). Control plants from high elevation had lower VOCs emissions than low-elevation plants ($F_{1,190} = 11.4$, $P = 0.001$). In contrast, when induced with jasmonate, we found no significant differences between high- and low-elevation ecotypes ($F_{1,190} = 0.05$, $P = 0.81$). We found similar results when the amount of emission from different RT were pooled into the total amount of VOCs produced, that is, a lower constitutive amount of VOCs produced by the high-elevation ecotype ($F_{1,8} = 16.7$, $P = 0.003$), but no significant difference after induction ($F_{1,8} = 0.37$, $P = 0.56$) (Fig. 5). Of particular note, the concentration of ocimene was twice as strong in the high compared with the low-elevation ecotype (Fig. 4).

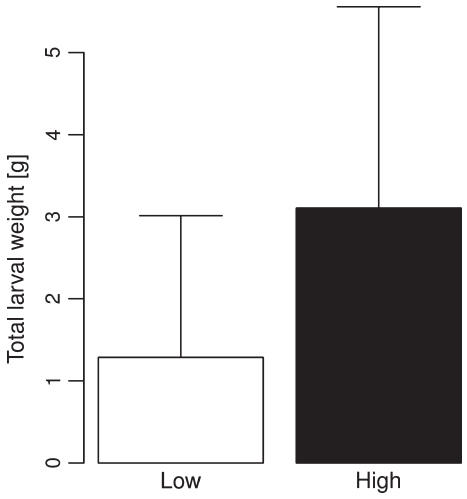

Fig. 3. Elevation effect on resistance in *Vicia sepium* plants. Shown are averages ±1 SD of the total larval weight of 30 larvae of *Spodoptera littoralis* placed during two weeks on individual of *Vicia sepium* plants that originated from low- (about 500 m) and high (about 1500 m) elevation collected seeds.

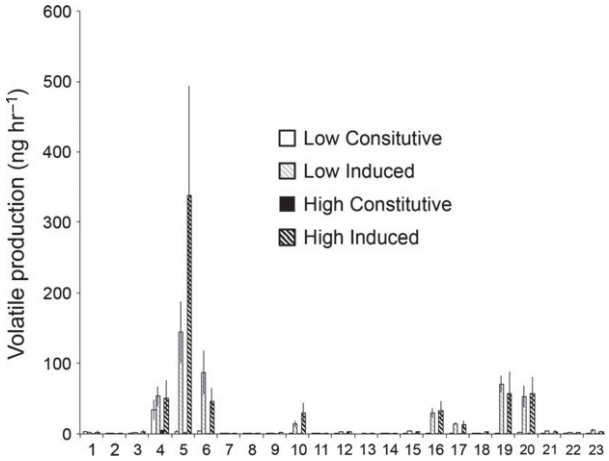

Fig. 4. Volatile organic compounds production of *Vicia sepium* plants. Shown are averages ±1 SD of constitutive (lighter colors) and jasmonic acid-induced (darker colors) volatiles of plants from low or high elevation. Numbers represent individual compounds: 1. Z,3-hexenol; 2. β-pinene; 3. β-myrcene; 4. unkonwn monoterpene; 5. ocimene; 6. unknown monoterpene; 7. linalool; 8. unknown monoterpene; 9. unknown compound; 10. methyl salicylate; 11. myrtenal; 12. jasmonol; 13. α-copaene; 14. unknown sesquiterpene; 15. β-caryophyllene; 16. α-caryophyllene; 17. β-farnesene; 18. – 21. unknown sesquiterpene; 22. nerolidol; 23. methyl-jasmonate. Identification of compounds is based on comparison of retention times and mass spectra with standards.

ANT ATTRACTION

The olfactometer experiment indicated a significant effect of induction with jasmonate on ant attraction (Fig. 6, $F_{1,84} = 9.0$, $P = 0.003$), no consistent effect of elevation ecotype ($F_{1,84} = 0.28$, $P = 0.75$), but a significant interaction between jasmonate induction and ecotype ($F_{1,84} = 4.75$, $P = 0.03$). First, the jasmonate treatment increased ant attraction to the plants. Second, jasmonate induction did not affect low- and high-elevation ecotypes in the same way. For the constitutive treatment, low-elevation ecotypes were more attractive than high-elevation ones. Once defences were induced, however, the high-elevation ecotypes were more attractive to ants. Because *V. sepium* is highly generalist in terms of the ant species that are attracted (see above results), we expected no effect of the ant species used in this experiment. Indeed, including ant species as a cofactor in the model did not influence the results.

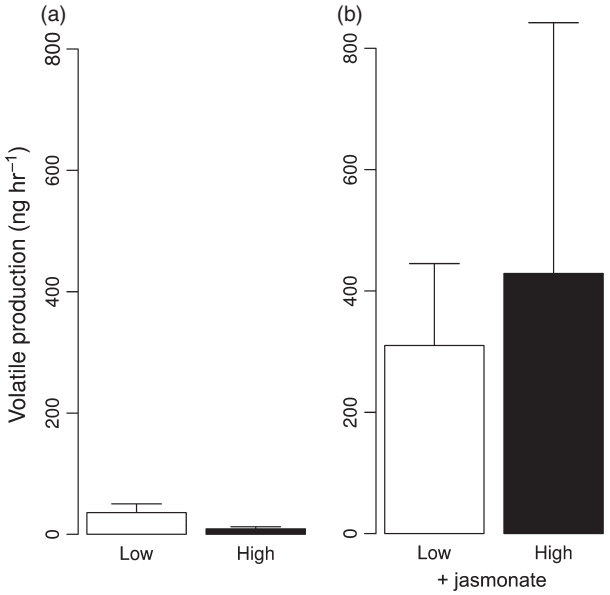

Fig. 5. Effect of elevation on volatile organic compounds (VOCs) production. Shown are averages ±1 SD of total VOCs produced by individual *Vicia sepium* plants from low- (about 500 m) or high (about 1500 m)-elevation collected seeds. VOCs were collected from healthy, undamaged plants (a), or after induction with methyl-jasmonate (b).

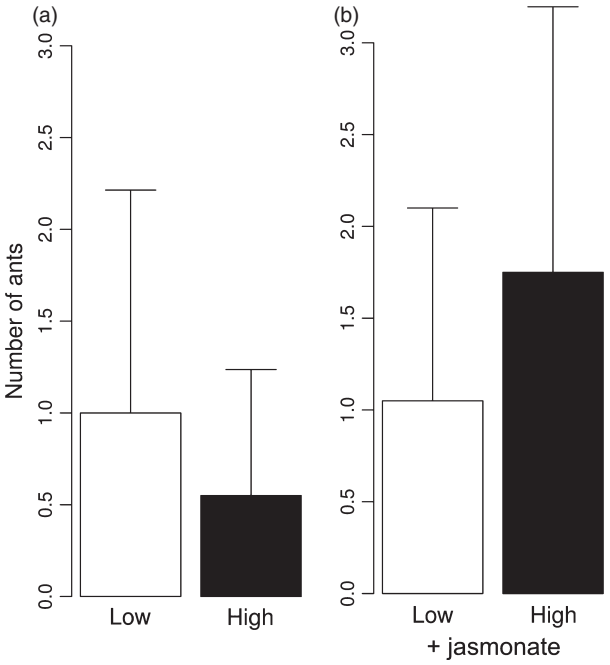

Fig. 6. Effect of plant ecotypes on ant recruitment. Shown are averages ±1 SD of ants that were attracted to *Vicia sepium* plants that originated from low- (about 500 m) or high-elevation (about 1500 m) collected seeds. Plants were either left undamaged (a), or induced with methyl-jasmonate (b).

Discussion

Our study is the first to simultaneously measure intraspecific variation in plant direct and indirect defence along elevation gradients. Consistent with a reduced investment in defence following the decrease in herbivory at higher elevations (Fig. 2c), we found lower plant resistance to herbivores as well as reduced constitutive VOCs emissions and EFNs production in the high-elevation ecotypes. These findings were coupled with an overall reduction in ant attendance in high-elevation populations (Fig. 2a). Once plant defences were induced with the wound-related hormone jasmonic acid; however, VOCs emissions were not different in high- and low-elevation populations. Moreover, ant attraction was enhanced in the high-elevation ecotype. Our results suggest that plants growing at high-elevation better modulate indirect defences. The benefits of defence mutualisms are expected to be context-dependent (Bronstein 1994). For instance, abiotic conditions should influence the costs and benefits of interaction in plant/ant mutualisms (Kersch & Fonseca 2005). In this context, we suggest that the potentially low-cost inducible defence strategy may be particularly suitable in environments where the herbivore composition and abundance are more variable in space and time and where the costs of establishing constitutive defence may be too high in the less productive environment (Agrawal & Karban 1999).

Spodopetra littoralis caterpillars experienced lower survival and grew significantly less rapidly on low-elevation ecotypes compared with high-elevation ecotypes growing in common garden conditions. These results show that direct defences (both constitutive and induced) are probably to be locally adapted to herbivore pressure, rather than purely the product of the available resources during the growing season. Supporting this result, a phylogenetically controlled experiment recently showed that high-elevation plants are less resistant than their congeneric low-elevation relatives (Pellissier *et al.* 2012a). Because insects are ectothermic, insect abundance and herbivory rates are generally expected to decrease with increasing elevation and thus modify the selective pressure on plant defence traits (Hodkinson 2005).

Components of the direct defence system should be responsible for the greater palatability of the high-elevation ecotype. We detected no cyanogenic glycosides in any of the plants analysed, but a change in concentration of other unmeasured molecules may shape differences in the direct defences of *V. sepium* from each elevation. For instance, nitrogen content has been shown to decrease in plant leaves at higher elevation (Dubuis *et al.* 2013). Therefore, lower palatability caused by reduced nitrogen fixing may explain the difference in survival between ecotypes, but this hypothesis requires further examination. In addition, it remains to be tested whether ecotypic differentiation between high- and low-elevation populations is the result of fixed genetic differences or whether resistance to herbivores is shaped by other non-genetic effects (Rasmann *et al.* 2013).

We also detected variation in indirect defence with elevation, but the pattern was more complex. Constitutively, high-elevation ecotypes produced significantly lower quantities of VOCs than low-elevation ones. However, both ecotypes produced a similar amount of VOCs when induced with methyl-jasmonate. Paralleling our results, Wason, Agrawal and Hunter (2013) recently showed that high-latitude populations

of *Asclepias syriaca* showed greater induction of VOCs emissions than low-latitude ones. However, using the same system, despite this difference in VOCs produced by plants from different latitudes, Wason and Hunter (2014) found no difference in recruitment of herbivore natural enemies between ecotypes. In contrast, here, we found increased ant attraction to the high-elevation ecotype of *V. sepium* following induction with methyl-jasmonate. Abundant evidence indicates that ants can be attracted by VOCs and EFNs (Brouat *et al.* 2000; Raguso 2004; Edwards *et al.* 2006; Kost & Heil 2008), but we further show that ant attraction levels may respond to phenotypic variation of plant ecotypes along ecological clines. Relatively few studies have reported intraspecific variation in VOCs emission in natural populations of plants and/or differences in the attraction of natural enemies (Kessler, Gase & Baldwin 2008; Kariyat *et al.* 2012; Pearse, Gee & Beck 2013; Wason & Hunter 2014). Yet, we know that the production of herbivore-induced VOCs depends on the interaction between biotic factors, including plant hormones (van Poecke & Dicke 2004), herbivore-derived elicitors (Halitschke *et al.* 2001), microorganisms (Huang *et al.* 2005) and abiotic factors, such as mechanical wounding (Mithofer, Wanner & Boland 2005), O_3 and CO_2 concentration (Jasoni *et al.* 2004; Vuorinen *et al.* 2004), temperature and light (Takabayashi, Dicke & Posthumus 1994; Gouinguene & Turlings 2002), UV-radiation (Johnson *et al.* 1999) and many other factors (Penuelas & Llusia 2001). We thus propose that modulation of herbivore enemy attraction may be an important element of plant adaptation to shifts in herbivore pressure and variation in environmental factors. Interestingly, we show that ocimene was produced at much higher concentrations in high- compared with low-elevation ecotypes. This monoterpene is known to be released by damaged plants (Halitschke *et al.* 2000) and to attract predators (Arimura *et al.* 2004; Shimoda *et al.* 2012). In future studies, this compound should be considered as a potential primary elicitor of ant attraction. Finally, it should be noted that VOCs production may be herbivore-specific and that induction may vary in response to attacks by particular herbivores (Pichersky, Noel & Dudareva 2006). Therefore, differences in VOCs profiles and concentrations between high- and low-elevation plants might have been stronger under real herbivore attacks compared with jasmonate induction.

Ant-mediated indirect defence, which reduces herbivory and increases fitness of the plant (Trager *et al.* 2010), can be triggered by the jasmonic acid signalling pathway (Radhika *et al.* 2008). However, the cost and benefit of constitutive relative to inducible defences may be context-dependent and, if so, the two strategies are expected to negatively correlate with each other (Agrawal, Conner & Rasmann 2010). Indeed, given the energetic cost of defences and the lower resource availability (e.g. growing season, nitrogen) at high compared with low elevation, redundant defence strategies should be counter-selected. Our results of low constitutive but high inducibility of VOCs production and ant attraction at high elevation, supports the hypothesis of habitat mediated trade-offs between defence strategies. Modulation of indirect defence may be a resource-efficient strategy for plants growing in environments where the probability of herbivore attack is reduced and more stochastic in space and time (Zangerl & Rutledge 1996). Future work is needed to disentangle the role of VOCs versus the role of nectar in mediating ant attraction (Raguso 2004; Kessler & Heil 2011). Yet, the observed ant attraction patterns to non-flowering plants suggest that VOCs are the predominant long-range attractant in the system. Following similar cost-based reasoning, direct and indirect defences should also trade-off, as was shown by Ballhorn *et al.* (2008) in lima bean plants. However, here, we show that both constitutive direct resistance and VOCs production (indirect defences) are reduced at high elevation. Similarly, it was shown that constitutive cardenolide production (direct defence) and VOCs production (indirect defence) in roots do not trade-off across genotypes of *Asclepias syriaca* (Rasmann *et al.* 2011). One possible explanation may be that the expected trade-off between direct and indirect defences is evident only after induction. Adjustments in indirect defences may also arise from differences in nectar production. On average, nectar production was 1.45 times higher in low-elevation plants compared with high-elevation ones ($F_{1,22} = 23.76$, $P < 0.0001$). Nevertheless, as we only surveyed one low- and one high-elevation population, difference in nectar production along elevation gradients requires further investigation.

We found a shift in ant species composition and diversity in the communities attending *V. sepium* populations along the elevation gradient. The number of ant species found in population of *V. sepium* was lower at low elevation, where the ant diversity is highest (Machac *et al.* 2011; Reymond *et al.* 2013). EFNs and higher nectar production are probably to be a better food source for ants at lower elevations and should be guarded by the more aggressive or specialized ant species. In contrast, because at high elevation, the nectar is a less reliable resource, *V. sepium* plants may attract a broader sample of ant species that are opportunistically taking advantage of EFNs when they are available. Indeed, our results support the hypothesis that high-elevation harsh climate and scattered resources correlate with a more generalist insect behaviour (Rasmann, Alvarez & Pellissier 2014), as recently found for ants associated with myrmecophilous butterflies (Pellissier *et al.* 2012b).

In summary, herbivore pressure, predator abundance and resource availability seem to be the main drivers of variation in investment into direct and indirect defences along elevation. The plants living in low-resource conditions at high elevation can re-allocate their resources in response to damage, but given the paucity of herbivores and the increased spatio-temporal variability in herbivore attacks, investment in inducible defences is a more favourable strategy. Therefore, plant-mediated multitrophic feedbacks between plants, herbivores and predators shape community structure along environmental gradients. In particular, decreases in the frequency of herbivores would reduce food supply for their predators both directly and indirectly via interactions with plants. Nevertheless, plants are able to mount higher levels of defences at

higher elevations when induced, as predicted by defence allocation hypothesis (Coley, Bryant & Chapin 1985; Zangerl & Bazzaz 1992).

Acknowledgements

We thank Matis Pellissier for help in the field and two anonymous reviewers who provided valuable comments to improve the manuscript. This manuscript is supported by a National Science Foundation Ambizione grant PZ00P3_131956/1 to S. R. L. P. was supported by the Danish FNU grant no 12-126430 and the 'Félix Bonjour' grant from the Société Académique Vaudoise. JP was supported by Swiss National Science Foundation grant 31003A_125306. We thank the communes of Bex and Lavey for providing the experimental sites.

References

Agrawal, A.A., Conner, J.K. & Rasmann, S. (2010) Tradeoffs and adaptive negative correlations in evolutionary ecology. *Evolution after Darwin: The First 150 Years* (eds M.A. Bell, D.J. Futuyma, W.F. Eanes & J.S. Levinton), pp. 243–268. Sinauer, Sunderland, MA, USA.

Agrawal, A.A. & Karban, R. (1999) Why induced defenses may be favored over constitutive strategies in plants. *The Ecology and Evolution of Inducible Defenses* (eds R. Tollrian & C.D. Harvell), pp. 45–61. Princeton University Press, Princeton, NJ, USA.

Arimura, G., Ozawa, R., Kugimiya, S., Takabayashi, J. & Bohlmann, J. (2004) Herbivore-induced defense response in a model legume. Two-spotted spider mites induce emission of (E)-beta-ocimene and transcript accumulation of (E)-beta-ocimene synthase in *Lotus japonicus*. *Plant Physiology*, **135**, 1976–1983.

Ballhorn, D.J., Kautz, S., Lion, U. & Heil, M. (2008) Trade-offs between direct and indirect defences of lima bean (*Phaseolus lunatus*). *Journal of Ecology*, **96**, 971–980.

Beattie, A.J. (1985) *The evolutionary ecology of ant-plant mutualisms*. Cambridge studies in ecology. Cambridge University Press, Cambridge, UK.

Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Korner, C., de Boeck, H., Christensen, J.H., Leuzinger, S., Janssens, I.A. & Hansen, K. (2012) Precipitation manipulation experiments - challenges and recommendations for the future. *Ecology Letters*, **15**, 899–911.

Brinker, A.M. & Seigler, D.S. (1992) Determination of cyanide and cyanogenic glycosides from plants. *Modern Methods of Plant Analysis New Series; Plant Toxin Analysis* (eds H.F. Linskens & J.F. Jackson), pp. 359–381. Springer, Berlin-Heidelberg, Germany.

Bronstein, J.L. (1994) Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, **9**, 214–217.

Brouat, C., McKey, D., Bessiere, J.M., Pascal, L. & Hossaert-McKey, M. (2000) Leaf volatile compounds and the distribution of ant patrolling in an ant-plant protection mutualism: preliminary results on *Leonardoxa* (*Fabaceae*: *Caesalpinioideae*) and *Petalomyrmex* (*Formicidae*: *Formicinae*). *Acta Oecologica-International Journal of Ecology*, **21**, 349–357.

Brown, E.S. & Dewhurst, C.F. (1975) The genus *Spodoptera* (*Lepidoptera*, *Noctuidae*) in Africa and the near east. *Bulletin of Entomological Research*, **65**, 221–262.

Coley, P.D., Bryant, J.P. & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science*, **230**, 895–899.

Darrow, K. & Bowers, M.D. (1997) Phenological and population variation in iridoid glycosides of *Plantago lanceolata* (*Plantaginaceae*). *Biochemical Systematics and Ecology*, **25**, 1–11.

Dicke, M. & Baldwin, I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, **15**, 167–175.

Dubuis, A., Rossier, L., Pottier, J., Pellissier, L., Vittoz, P. & Guisan, A. (2013) Predicting current and future spatial community patterns of plant functional traits. *Ecography*, **36**, 1158–1168.

Edwards, D.P., Hassall, M., Sutherland, W.J. & Yu, D.W. (2006) Assembling a mutualism: ant symbionts locate their host plants by detecting volatile chemicals. *Insectes Sociaux*, **53**, 172–176.

Farmer, E.E. & Ryan, C.A. (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *Plant Cell*, **4**, 129–134.

Fine, P.V.A., Mesones, I. & Coley, P.D. (2004) Herbivores promote habitat specialization by trees in Amazonian forests. *Science*, **305**, 663–665.

de la Fuente, M.A.S. & Marquis, R.J. (1999) The role of ant-tended extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. *Oecologia*, **118**, 192–202.

Gouinguene, S.P. & Turlings, T.C.J. (2002) The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, **129**, 1296–1307.

Halitschke, R., Kessler, A., Kahl, J., Lorenz, A. & Baldwin, I.T. (2000) Ecophysiological comparison of direct and indirect defenses in *Nicotiana attenuata*. *Oecologia*, **124**, 408–417.

Halitschke, R., Schittko, U., Pohnert, G., Boland, W. & Baldwin, I.T. (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (*Lepidoptera*, *Sphingidae*) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiology*, **125**, 711–717.

Heil, M. (2008) Indirect defence via tritrophic interactions. *New Phytologist*, **178**, 41–61.

Heil, M., Fiala, B., Maschwitz, U. & Linsenmair, K.E. (2001) On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia*, **126**, 395–403.

Heil, M., Greiner, S., Meimberg, H., Kruger, R., Noyer, J.L., Heubl, G., Linsenmair, K.E. & Boland, W. (2004) Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature*, **430**, 205–208.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants - to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.

Hodkinson, I.D. (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, **80**, 489–513.

Holldobler, B. & Wilson, E.O. (1990) *The Ants*. Belknap Press of Harvard University Press, Cambridge, MA, USA.

Howe, G.A. & Jander, G. (2008) Plant immunity to insect herbivores. *Annual Review of Plant Biology*, **59**, 41–66.

Huang, J., Schmelz, E.A., Alborn, H., Engelberth, J. & Tumlinson, J.H. (2005) Phytohormones mediate volatile emissions during the interaction of compatible and incompatible pathogens: the role of ethylene in *Pseudomonas syringae* infected tobacco. *Journal of Chemical Ecology*, **31**, 439–459.

Jasoni, R., Kane, C., Green, C., Peffley, E., Tissue, D., Thompson, L., Payton, P. & Pare, P.W. (2004) Altered leaf and root emissions from onion (*Allium cepa* L.) grown under elevated CO_2 conditions. *Environmental and Experimental Botany*, **51**, 273–280.

Johnson, C.B., Kirby, J., Naxakis, G. & Pearson, S. (1999) Substantial UV-B-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). *Phytochemistry*, **51**, 507–510.

Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. The University of Chicago Press, Chicago.

Kariyat, R.R., Mauck, K.E., De Moraes, C.M., Stephenson, A.G. & Mescher, M.C. (2012) Inbreeding alters volatile signalling phenotypes and influences tri-trophic interactions in horsenettle (*Solanum carolinense* L.). *Ecology Letters*, **15**, 301–309.

Kersch, M.F. & Fonseca, C.R. (2005) Abiotic factors and the conditional outcome of an ant-plant mutualism. *Ecology*, **86**, 2117–2126.

Kessler, A. & Baldwin, I.T. (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**, 2141–2144.

Kessler, D., Gase, K. & Baldwin, I.T. (2008) Field experiments with transformed plants reveal the sense of floral scents. *Science*, **321**, 1200–1202.

Kessler, A. & Heil, M. (2011) The multiple faces of indirect defences and their agents of natural selection. *Functional Ecology*, **25**, 348–357.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution*, **22**, 569–574.

Kost, C. & Heil, M. (2008) The defensive role of volatile emission and extrafloral nectar secretion for lima bean in nature. *Journal of Chemical Ecology*, **34**, 2–13.

Machac, A., Janda, M., Dunn, R.R. & Sanders, N.J. (2011) Elevational gradients in phylogenetic structure of ant communities reveal the interplay of biotic and abiotic constraints on diversity. *Ecography*, **34**, 364–371.

Mithofer, A., Wanner, G. & Boland, W. (2005) Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology*, **137**, 1160–1168.

Novotny, V., Miller, S.E., Basset, Y., Cizek, L., Darrow, K., Kaupa, B., Kua, J. & Weiblen, G.D. (2005) An altitudinal comparison of caterpillar (*Lepidoptera*) assemblages on Ficus trees in Papua New Guinea. *Journal of Biogeography*, **32**, 1303–1314.

Pearse, I.S., Gee, W.S. & Beck, J.J. (2013) Headspace volatiles from 52 oak species advertise induction, species identity, and evolution, but not defense. *Journal of Chemical Ecology*, **39**, 90–100.

Pellissier, L., Rasmann, S., Litsios, G., Fiedler, K., Dubuis, A., Pottier, J. & Guisan, A. (2012a) High host-plant nitrogen content: a prerequisite for the

evolution of ant–caterpillar mutualism? *Journal of Evolutionary Biology*, **25**, 1658–1666.

Pellissier, L., Fiedler, K., Ndribe, C., Dubuis, A., Pradervand, J.-N., Guisan, A. & Rasmann, S. (2012b) Shifts in species richness, herbivore specialization, and plant resistance along elevation gradients. *Ecology and Evolution*, **2**, 1818–1825.

Penuelas, J. & Llusia, J. (2001) The complexity of factors driving volatile organic compound emissions by plants. *Biologia Plantarum*, **44**, 481–487.

Pichersky, E., Noel, J.P. & Dudareva, N. (2006) Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science*, **311**, 808–811.

Pickett, S.A. (1989) Space-for-time substitution as an alternative to long-term studies. *Long-Term Studies in Ecology* (ed. G. Likens), pp. 110–135. Springer, New York.

van Poecke, R.M.P. & Dicke, M. (2004) Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. *Plant Biology*, **6**, 387–401.

Preszler, R.W. & Boecklen, W.J. (1996) The influence of elevation on tri-trophic interactions: opposing gradients of top-down and bottom-up effects on a leaf-mining moth. *Ecoscience*, **3**, 75–80.

Radhika, V., Kost, C., Bartram, S., Heil, M. & Boland, W. (2008) Testing the optimal defence hypothesis for two indirect defences: extrafloral nectar and volatile organic compounds. *Planta*, **228**, 449–457.

Raguso, R.A. (2004) Why are some floral nectars scented? *Ecology*, **85**, 1486–1494.

Rasmann, S., Alvarez, N. & Pellissier, L. (2014) The altitudinal niche breadth hypothesis in plant-insect interaction. *Annual Plant Reviews* (eds C. Voelckel & G. Jander), pp. 339–360. Wiley, Hoboken, NJ, USA.

Rasmann, S., Kollner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J. & Turlings, T.C.J. (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature*, **434**, 732–737.

Rasmann, S., Erwin, A.C., Halitschke, R. & Agrawal, A.A. (2011) Direct and indirect root defences of milkweed (*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. *Journal of Ecology*, **99**, 16–25.

Rasmann, S., Pellissier, L., Defossez, E., Jactel, H. & Kunstler, G. (2013) Climate-driven change in plant–insect interactions along elevation gradients. *Functional Ecology*, **28**, 46–54.

Reymond, A., Purcell, J., Cherix, D., Guisan, A. & Pellissier, L. (2013) Functional diversity decreases with temperature in high elevation ant fauna. *Ecological Entomology*, **38**, 364–373.

Rodriguez-Castaneda, G., Forkner, R.E., Tepe, E.J., Gentry, G.L. & Dyer, L.A. (2011) Weighing defensive and nutritive roles of ant mutualists across a tropical altitudinal gradient. *Biotropica*, **43**, 343–350.

Rodríguez-Castañeda, G., Dyer, L.A., Brehm, G., Connahs, H., Forkner, R.E. & Walla, T.R. (2010) Tropical forests are not flat: how mountains affect herbivore diversity. *Ecology Letters*, **13**, 1348–1357.

Salmore, A.K. & Hunter, M.D. (2001) Elevational trends in defense chemistry, vegetation, and reproduction in *Sanguinaria canadensis*. *Journal of Chemical Ecology*, **27**, 1713–1727.

Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M. (2005) *Insect-plant biology*. Oxford University Press, Oxford.

Schuman, M.C., Barthel, K. & Baldwin, I.T. (2012) Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature. *eLife Sciences*, **1**, e00007.

Shimoda, T., Nishihara, M., Ozawa, R., Takabayashi, J. & Arimura, G.-I. (2012) The effect of genetically enriched (E)-beta-ocimene and the role of floral scent in the attraction of the predatory mite *Phytoseiulus persimilis* to spider mite-induced volatile blends of torenia. *New Phytologist*, **193**, 1009–1021.

Singer, M.S. & Stireman, J.O. (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. *Ecology Letters*, **8**, 1247–1255.

Stephenson, A.G. (1982) The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology*, **63**, 663–669.

Strong, D.R., Lawton, J.H. & Southwood, R. (1984) *Insects on plants: community patterns and mechanisms*. Blackwell Scientific, London, U.K.

Takabayashi, J., Dicke, M. & Posthumus, M.A. (1994) Volatile herbivore-induced terpenoids in plant-mite interactions: variation caused by biotic and abiotic factors. *Journal of Chemical Ecology*, **20**(6), 1329–1354.

Thaler, J.S. & Karban, R. (1997) A phylogenetic reconstruction of constitutive and induced resistance in *Gossypium*. *American Naturalist*, **149**, 1139–1146.

Trager, M.D., Bhotika, S., Hostetler, J.A., Andrade, G.V., Rodriguez-Cabal, M.A., McKeon, C.S., Osenberg, C.W. & Bolker, B.M. (2010) Benefits for plants in ant-plant protective mutualisms: a meta-analysis. *PLoS ONE*, **5**, e14308.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, **250**, 1251–1253.

Turlings, T.C.J. & Wäckers, F.(2002) Recruitment of predators and parasitoids by herbivore-injured plants. Advances in Chemical Ecology (eds R.T. Cardé & J.G. Millar), pp. 21–75. Cambridge University Press, West Nyack, NY, USA.

Vuorinen, T., Nerg, A.M., Ibrahim, M.A., Reddy, G.V.P. & Holopainen, J.K. (2004) Emission of *Plutella xylostella*-induced compounds from cabbages grown at elevated CO_2 and orientation behavior of the natural enemies. *Plant Physiology*, **135**, 1984–1992.

Wason, E.L., Agrawal, A.A. & Hunter, M.D. (2013) A genetically-based latitudinal cline in the emission of herbivore-induced plant volatile organic compounds. *Journal of Chemical Ecology*, **39**, 1101–1111.

Wason, E. & Hunter, M. (2014) Genetic variation in plant volatile emission does not result in differential attraction of natural enemies in the field. *Oecologia*, **174**, 479–491.

Wink, M. (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, **64**, 3–19.

Zangerl, A.R. & Bazzaz, F.A. (1992) Theory and pattern in plant defense allocation. *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution and Genetics* (eds R.S. Fritz & E.L. Simms), pp. 363–391. University of Chicago Press, Chicago.

Zangerl, A.R. & Rutledge, C.E. (1996) The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *American Naturalist*, **147**, 599–608.

Zehnder, C.B., Stodola, K.W., Joyce, B.L., Egetter, D., Cooper, R.J. & Hunter, M.D. (2009) Elevational and seasonal variation in the foliar quality and arthropod community of *Acer pensylvanicum*. *Environmental Entomology*, **38**, 1161–1167.